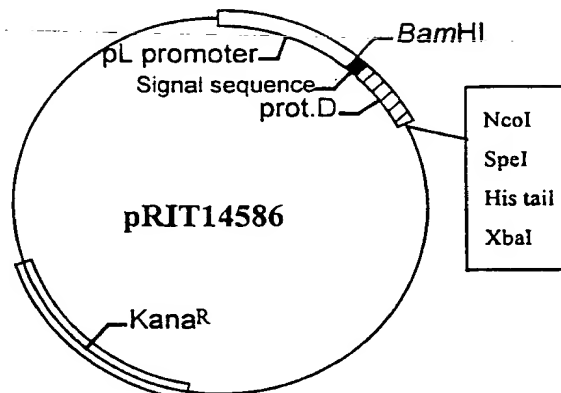


Figure 1: A/ Map of plasmid pRIT14586

B/ Coding sequence of the first 127 amino acids
of protein D and multiple cloning site. The signal
sequence is underlined.

BamHI
ATG GAT CCA AAA ACT TTA GCC CTT TCT TTA TTA GCA GCT GGC GTA CTA GCA GGT TGT AGC AGC
Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Leu Ala Ala Gly Val Leu Ala Gly Cys Ser Ser
CAT TCA TCA AAT ATG GCG AAT ACC CAA ATG AAA TCA GAC AAA ATC ATT ATT GCT CAC CGT GGT
His Ser Ser Asn Met Ala Asn Thr Gln Met Lys Ser Asp Lys Ile Ile Ile Ala His Arg Gly
GCT AGC GGT TAT TTA CCA GAG CAT ACG TTA GAA TCT AAA GCA CTT GCT TTT GCA CAA CAG GCT
Ala Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gln Gln Ala
GAT TAT TTA GAG CAA GAT TTA GCA ATG ACT AAG GAT GGT CGT TTA GTG GTT ATT CAC GAT CAC
Asp Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val Ile His Asp His
TTT TTA GAT GGC TTG ACT GAT GTT GCG AAA AAA TTC CCA CAT CGT CAT CGT AAA GAT GGC CGT
Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe Pro His Arg His Arg Lys Asp Gly Arg
TAC TAT GTC ATC GAC TTT ACC TTA AAA GAA ATT GAA AGT TTA GAA ATG ACA GAA AAC TTT GAA
Tyr Tyr Val Ile Asp Phe Thr Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu
NcoI ACC ATG GCC ACG TGT GAT CAG AGC TCA SpeI ACT AGT GGA CAC CAT CAC CAT CAC CAT TAA TCT AGA XbaI
Thr Met Ala Thr Cys Asp Gln Ser Ser Thr Ser Gly His His His His His His *

The amino acid sequence of Figure 1 relates to Seq. ID no. 7 and the nucleic acid sequence of
Figure 1 relates to Seq. ID. No. 6.

The DNA and amino acid sequences of Nef-His; Tat-His; Nef-Tat-His fusion and mutated Tat is illustrated.

Pichia-expressed constructs (plain constructs)

⇒ Nef - HIS

DNA sequence (Seq. ID. No. 8)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGC
CACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 9)

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFVPTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ Tat - HIS

DNA sequence (Seq. ID. No. 10)

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAA
ACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTC
ATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
CCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAA

09509239.032300

TCCCGAGGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence (Seq. ID. No. 11)

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRR
PPQGSQTHQVLSKQPTSQSRGDPTGPKETSGHHHHHH.

⇒ Nef - Tat - HIS

DNA sequence (Seq. ID. No. 12)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAG
CCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAAGTCT
TGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTCATAACA
AAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCT
CAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGA
GGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 13)

^^
MGGKWSKSSVVGWPTVVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTA
CTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVLSKQPTSQSR
GDPTGPKETSGHHHHHH.

E.coli-expressed constructs (fusion constructs)

⇒ LipoD-Nef-HIS

DNA sequence (Seq. ID. No. 14)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
 AGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
 GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
 GCTTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
 CGTTTAGTGGTTATTCACGATCACTTTTATAGATGGCTTGACTGATGTTGCGAAAAA
 TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
 GAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGCAAGTGGTCA
 AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
 GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
 AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
 GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
 GCAGCTGTAGATCTTAGCCACTTTTTAAAGAAAAAGGGGGGACTGGAAGGGCTAATT
 CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
 TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTT
 GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
 GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
 AGAGAAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCCG
 GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCACCATCACCAT
 TAA

Protein sequence of the processed lipidated ProtD-Nef-HIS protein (Seq. ID. No. 15)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMTKD
 GRLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGGKW
 SKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
 EEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWYHTQG
 YFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLHGMDDP
 EREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ LipoD-Nef-Tat-HISDNA sequence (Seq. ID. No. 16)

00509239-03200

Nucleotides corresponding to the Prot D Fusion Partner are in bold.
The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
AGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACTGTTAGAATCTAAAGCACTT
GCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
CGTTTAGTGTTATTACGATCACTTTTTAGATGGCTTGACTGATGTTGCGAAAAAA
TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
GAAATTCAAAGTTTAGAAATGACAGAAAACCTTTGAAACCATGGGTGGCAAGTGGTCA
AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
GCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATT
CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTT
GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCGA
GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTA
GAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTATTGT
AAAAAGTGTGTCTTTTATTGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCC
TATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCAT
CAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCG
AAGGAAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence of the processed lipidated ProtD-NEF-TAT-HIS protein (Seq. ID. No. 17)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQQADYLEQDLAMTKD
 GRLVVIHDFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMENFETMGGKW
 SKSSVVGWPTVRERMRRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
 EEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQG
 YFPDWQNYTPGPGVRYPLTFGWICYKLVPEPDKVEEANKGENTSLLHPVSLHGMDDP
 EREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCY
 CKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTG
 PKETSGHHHHHHH.

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⇒ ProtD-Nef-HISDNA sequence (Seq. ID. No. 18)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
 ATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
 AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
 AAGGATGGTCGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
 GCGAAAAAATTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
 ACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACCTTGAAACCATGGGTGGC
 AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
 GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
 GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
 CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
 ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAA
 GGGCTAATCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
 CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCA
 CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
 GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
 GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAC
 GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCAC
 CATCACCATTAA

Protein sequence (Seq. ID. No. 19)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIAHRGASGYLPEHTLESKALAFQAQADYL
 EQDLAMTKDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLK
 EIQSLEMTENFETMGGKWSKSSVVGWPTVRERMRRAPAADGVGAASRDL
 EKHGAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSH
 FLKEKGGLEGLIHSQRRQDILDWYHTQGYFPDWQNYTPGPGVRYPLTFGW
 CYKLVPVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFDSRLAFH
 HVARELHPEYFKNCTSGHHHHHH.

⇒ ProtD-Nef-Tat-HISDNA sequence (Seq. ID. No. 20)

00509239-032300

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Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACC GTGGT GCTAGCGGTTATTTACCAGAGCATA CGTTAG AATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTT AGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCACACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACCTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAA
GGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCA
CTGACCTTTGGATGGTGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGAT
CCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAAT
TGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTTCATAACAAAAGCCTTA
GGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGT
CAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCG
ACAGGCCCGAAGGAAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 21)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMT
KDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGG
KWSKSSVVGWPTVYRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEA
QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWLYHT
QGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLHGMD
DPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTN
CYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDP
TGPKETSGHHHHHH.

⇒ Tat-MUTANT-HIS

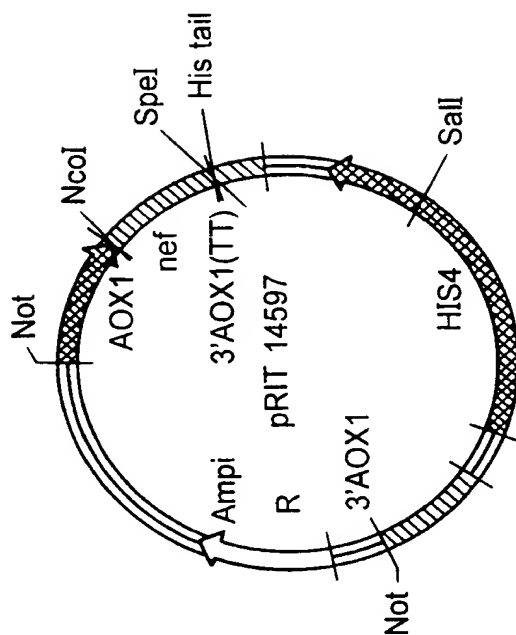
DNA sequence (Seq. ID. No. 22)

Protein sequence (Seq. ID. No. 25)

MGGKWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKH	40
GAITSSNTAATNAACAWLEAQEEEEVGFVPVPQVPLRPMT	80
YKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLIWYHTQGY	120
FPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGE	160
NTSLLHPVSLHGMDDPEREVLEWRFDSSLAFHHVARELHP	200
EYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFH	240
CQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQP	280
TSQSKGEPTGPKETSGHHHHHH.	302

SECRET

Fig. 3 Map of pRIT14597 integrative vector

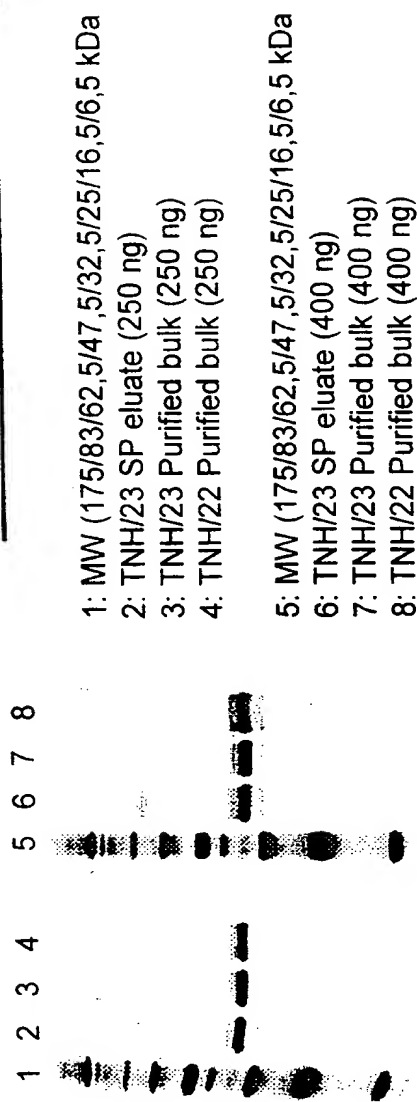


MCS POLYLINKER: *nef* gene inserted between NcoI and SpeI sites.

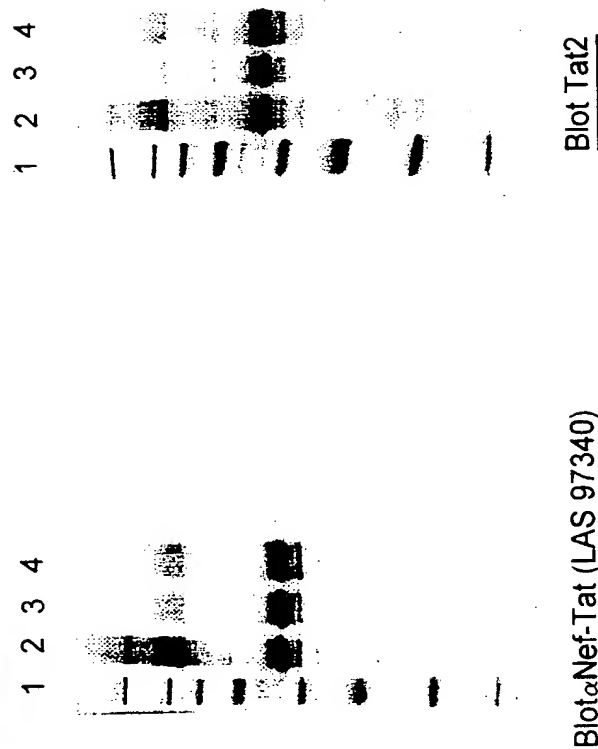
<i>Acu II</i>	<i>Nco I</i>	<i>Spe I</i>	<i>Eco RI</i>
<u>TTCGAA</u>	<u>ACC.ATGGCCGCGG</u>	<u>ACTAGT</u>	<u>GGC.CAC.CAT.CAC.CAT.TAA.CGGAATTC</u>
		Thr . Ser . Gly .	His . His . His . His . His

The amino acid sequence of Figure 3 relates to Seq. ID no. 27 and the nucleic acid sequence of Figure 3 relates to Seq. ID. No.26.

Fig. 4 SDS-PAGE: Nef-Tat-his fusion protein

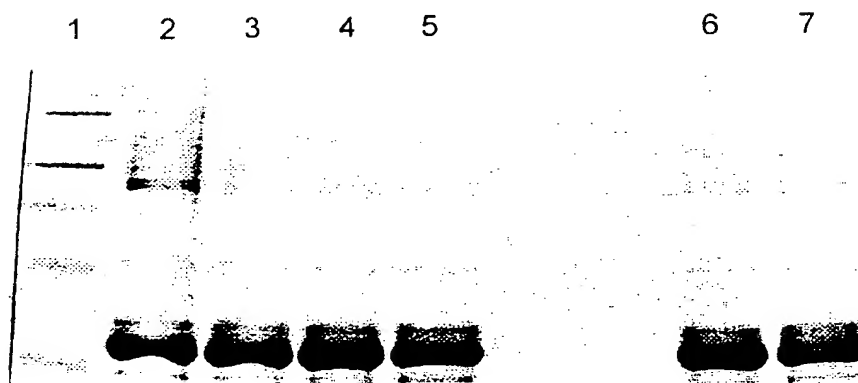


Daiichi Silver Staining

Blot α Nef-Tat (LAS 97340)

Blot Tat2

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Fig . 5 SDS-PAGE: Nef-Tat-his fusion proteinCoomassie blue G250

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa)
- 2: TNH/23 SP eluate (4 μ g)
- 3: TNH/23 Superdex200 eluate (4 μ g)
- 4: TNH/23 Purified bulk (4 μ g)
- 5: TNH/22 Purified bulk (4 μ g)

- 6: TNH/23 Purified bulk (4 μ g) / non reducing conditions
- 7: TNH/22 Purified bulk (4 μ g) / non reducing conditions

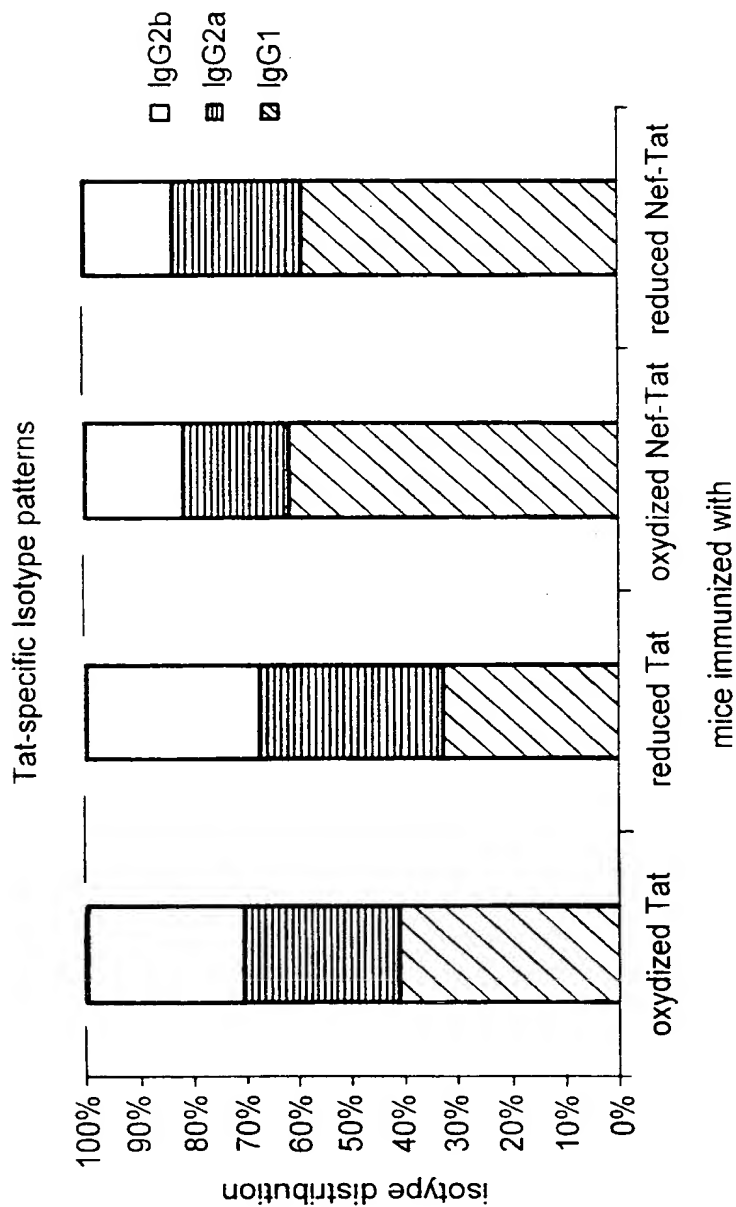
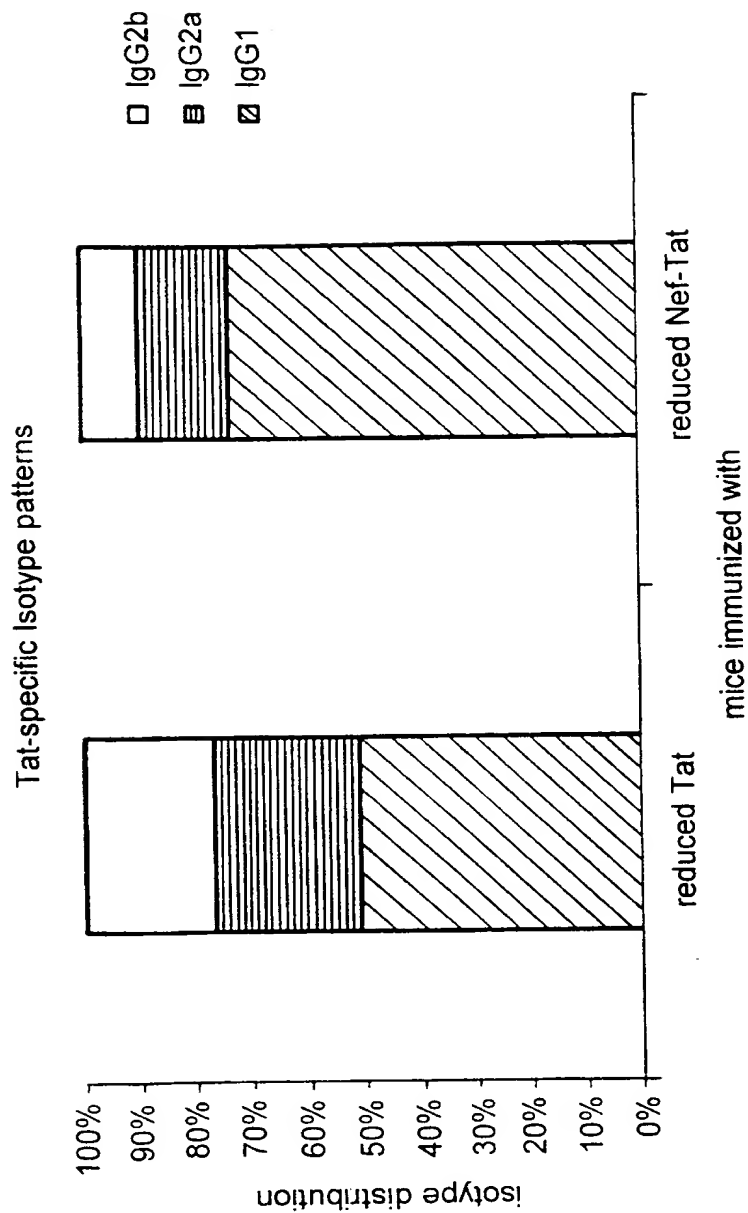


Fig. 6B

group	immunization	midpoint titers				
		lg	lgG1	lgG2a	lgG2b	ratio lgG1/lgG2a
1	reduced Tat	212799	123242	62697	55763	1,966
2	reduced Nef-Tat	75676	84046	18449	11692	4,556
3	adjuvant only	<4000	<4000	<4000	<4000	



002260-62260560

Fig. 7 Antigen-specific lymphoproliferative response of pooled lymph node cells

[3H] Thymidine incorporation in cpm				Data expressed as stimulation index			
Group 1 reduced Tat		Group 2 reduced Nef-Tat		Group 1 reduced Tat		Group 2 reduced Nef-Tat	
reduced Tat 5µg/ml 1µg/ml 0.2µg/ml	41967	18511	789	140	115	1	1
	37609	32346	415	125	201	1	1
	27640	23408	397	92	145	1	1
reduced Nef-Tat 5µg/ml 1µg/ml 0.2µg/ml	43882	31694	483	146	197	1	1
	33865	28094	245	113	174	0	0
	25079	22891	383	84	142	1	1
medium		161	571	1	1	1	1

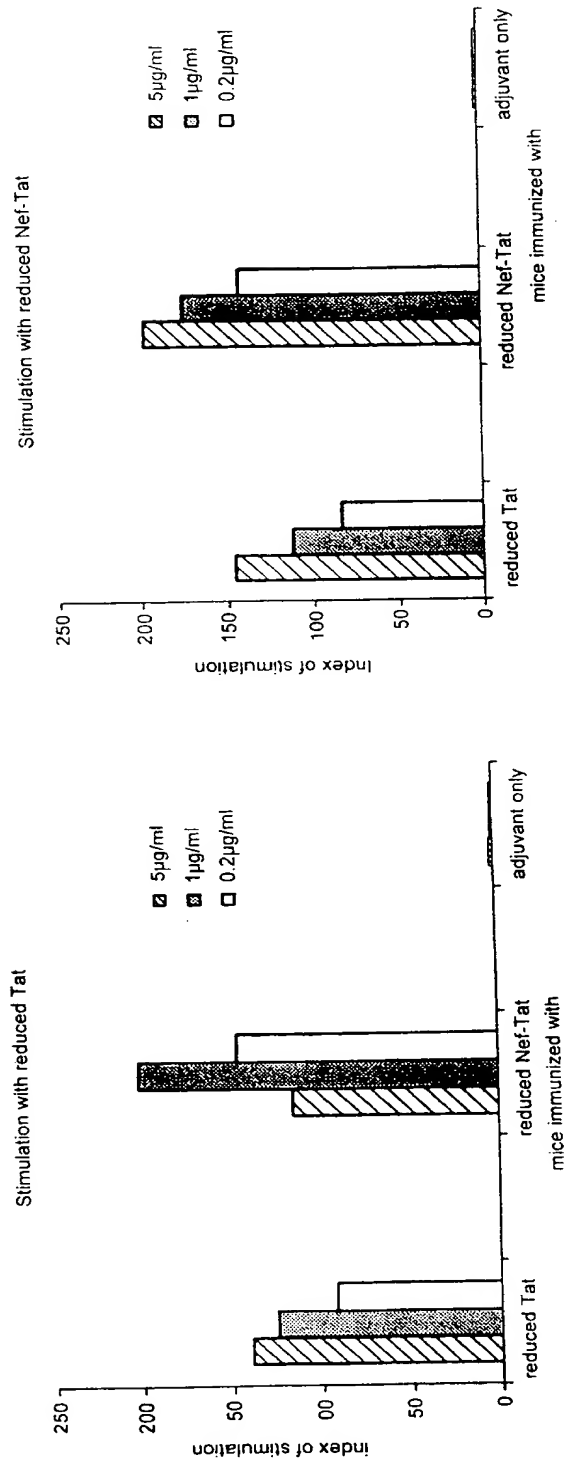


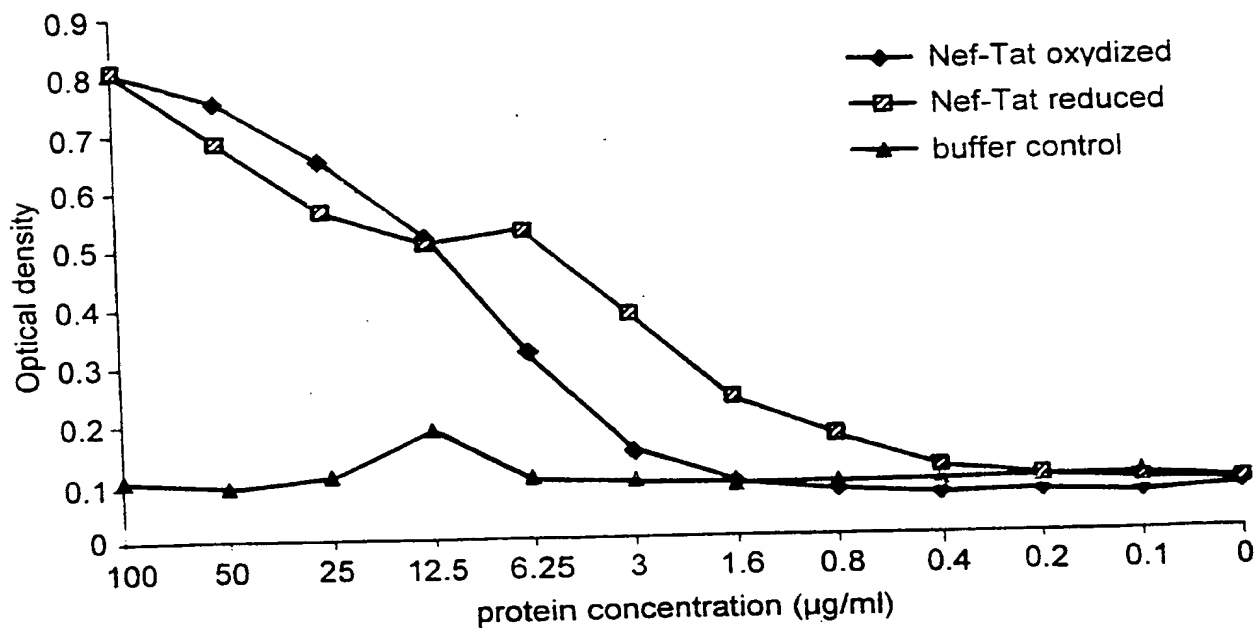
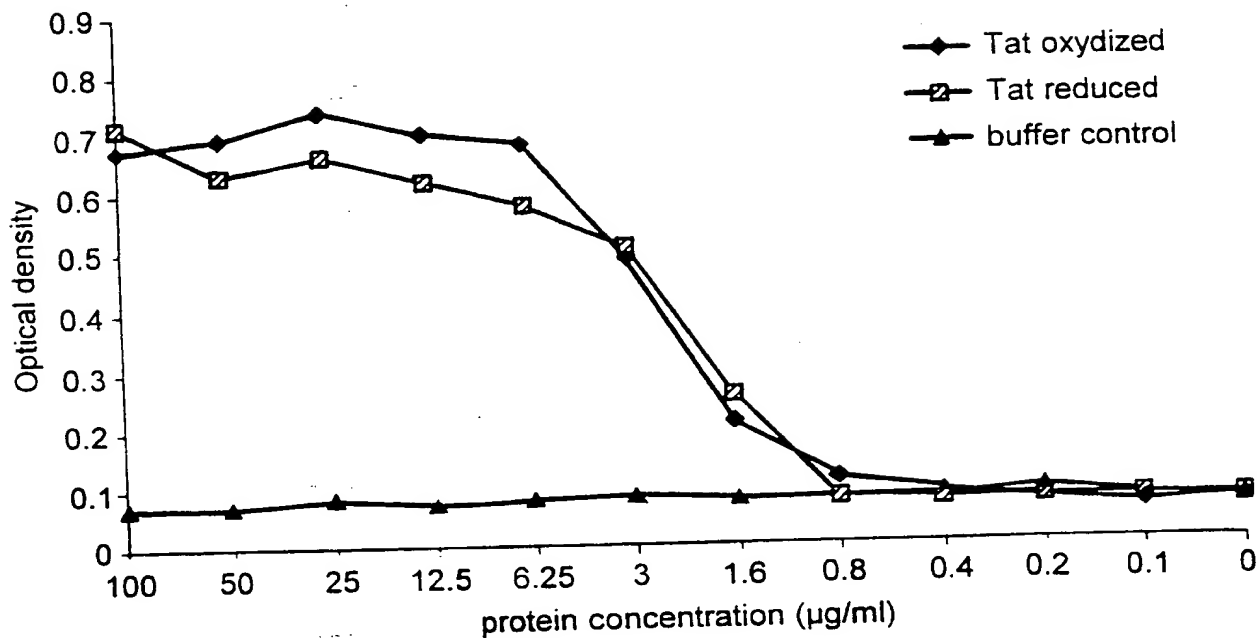
Fig. 8 Cell binding assay

Fig. 9 Inhibition of cell growth